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SODIUM FLUORIDE INDUCED CHANGES IN THE GROWTH AND YIELD OF *SPINACIA OLERACEA* L.

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ABSTRACT: The growing rate of globalization, industrialization and the resultant population growth (urbanization) have caused severe environmental problems in the world. One of these problems is soil salinity; a threat to crop production in Pakistan. Fluoride acts as an environmental pollutant and even at lower ambient fluoride concentrations many physiological changes may begin in the plants. Some of these changes may have important consequences such as reduction in growth and yield. Therefore, the present investigation was commenced to determine the responses of Spinach plants grown under fluoride stress given. The effect of Sodium Fluoride on Spinach variety (All Green) was studied during growth season October 2019-December 2019. The concentrations of fluoride of 0, 50, 100, 150, 200, 250, and 300 ppm were applied to plants as soil drench twice a week to create stressed condition. It was observed that plant height and biomass production of Spinach plant decreased under Fluoride stress while this stress was not found in control. Successive gradual reduction in yield characters was also observed with increased Sodium Fluoride concentration. After 60 days of sowing, shoot and root length of plants treated with 300 ppm were 16.23 cm and 7.53 cm respectively, far less than those of control. By observing the deleterious effects of NaF on plants, it is concluded that sodium fluoride present in the soil causes a marked reduction in growth and other attributes of Spinach plant.

Keywords: Fluoride stress; Growth; *Spinacia oleracea* L.; Yield

INTRODUCTION

During the recent era of climate change, salt stress is a perilous threat to crop production [1]. Plant performance is mainly hindered by salt stress. Every year a huge damage of $27.3 billion is incurred to agriculture globally [2]. Plant productivity has been lost by 50% throughout the world [3]. Immoderate levels of salt primarily impose damaging effects on plant development by exerting osmotic and some ionic impacts [4]. Solute concentration, plant growth stage and stress severity are some of the factors that determine the complex response of plants towards salinity [5]. In recent times, arid and semiarid regions having little rainfall and uncurbed rate of evapotranspiration are specially being deleteriously affected in terms of their agricultural productivity [6]. Predictions for 50% arable land are there to be left unproductive by 2050 due to high levels of salt [7]. This trend leads to a tough challenge of meeting all food needs globally, so during this challenging situation the increase of crop productivity in arable and even in infertile land is the need of hour [8].

Almost all the earth crust contains fluoride element which is at number 13 with respect to its abundance in earth and extensively found in pedosphere. Irrigation, over

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application of phosphorus containing fertilizers and aluminum extraction are some of the major industrial activities contributing toward high F composition in soil [9]. Wastewater released from industries such as fertilizers, production of insecticides, electronics, aluminium and glass semiconductors also adds fluoride into the environment [10]. So plants take excessive fluorides up from soil. Plant growth rate and biomass are adversely affected due reduction in photosynthetic pigments, along with symptoms of chlorotic and necrotic leaves [11]. Disruption in ion homeostasis and enzyme activity is cause by fluoride toxicity in environment [12].

Regardless of whether or not F is an essential trace element in humans, F is considered to be toxic when its level is high [13,14]. Plants have evolved mechanisms of self-defence for balanced homeostasis against any environmental (F stress) or genetic factor [15]. F toxicity causes intense and quick symptoms in highly sensitive plants [16,17]. From atmosphere, F can directly enter in plants majorly through stomata or to a lower extent through leaf cuticle [18]. Once it is up taken by plant, it can move throughout the plant via xylem tissues either by apoplastic or by simplistic pathway [19]. After getting inside the cell, physiological disturbance in plant cells leads to abnormal growth and development of plant [20].

Spinacia oleracea L. is a member of Amaranthaceae family. This edible flowering plant is natively grown in Asia's central and southwestern region [21]. Presence of different antioxidants, proteins, omega-3, fatty acids and vitamins A, C, E, K, B2, B6 make it very nutritious vegetable that is also rich in different ions and minerals [22]. It also ensures provision of glucuronic acid derivatives of flavonoids and derivatives of p-coumaric acid, both play role of significantly important antioxidants [23].

Pakistan has spinach as a major grown vegetable in all the regions of country. Forty-one calories are obtained by 200g of spinach and that is why it is widely cultivated here [24]. Spinach is a green vegetable. This leafy vegetable is used as a super nutritious food. Its leafy parts accumulate a very significant amount of heavy metals and some other toxic ions in it, which makes it more sensitive to some toxic pollutants than other nonleafy vegetables [25]. In Pakistan due to its medicinal value and nutritional importance, spinach is a valuable crop. Therefore, Spinach (*Spinacia oleraecea* L) was chosen to study F uptake, its toxicity and changes in various growth parameters and productivity, grown under NaF stress.

MATERIALS AND METHODS

Plant materials and experimental site: Healthy and authorized hybrid seeds of Spinach (*Spinacia oleracea* L.), variety "All Green", having F_1 generation were acquired from Roshan Seed Corporation, Lahore. Seeds were healthy greenish and crinkly. The location of experimental site is at the south of the city (74º 21-00-E, 31º 35-00-N) Botanical Garden, University of the Punjab, Department of the Botany, Quaid-e-Azam Campus, Lahore. Field work was conducted from October, 2019 to December, 2019. To avoid influence of different pests i.e. Birds, Mongoose and other rodents such as Squirrels and effect of heavy rain fall, all the experimental assembly was enclosed in a wire house.

Soil and pot preparation: Standard agricultural plan of action was adopted while preparing soil for plant production. Sandy loamy soil was prepared with a ratio of 1:3 and farm manure was also added into the soil to enhance its fertility. Forty-two clay pots were arranged according to randomized complete block design (RCBD). Two

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harvests were taken: first and final. Each treatment had 6 replicates, i.e., 3 replicates for each harvest. All the pots were tagged regarding to their respective treatments and replicate number.

Preparation of solutions: Solutions of 6 different concentrations were prepared by adding measured weight of sodium fluoride (NaF) salt in distilled water. The solutions (treatments) of different concentrations that were applied during experiment are 0, 50, 100, 150, 200, 250 and 300 ppm.

Raising of seedlings and application of sodium fluoride: Soaking of seeds was not required as the seeds were too small in size and also their seed coat was not too much hard. The germination was completed 10 DAS. Then thinning was performed when the plants had grown up enough to withstand any environmental stress. Sodium Fluoride solutions were applied as soil drench. After 28 days of sowing, first treatment was given. The concentrations of NaF solutions were 50, 100, 150, 200, 250, 300 ppm. Each pot was provided with 100mL of NaF solution.

Determination of morphological parameters: To check the response of plants towards different concentrations of Sodium Fluoride stress, different morphological parameters that were measured: Length of shoot (cm), Length of root (cm), Leaf Area (cm^2), Number of Leaves and Petiole length. Formula derived by Carleton and Foote [26] in 1965 was used to calculate the area of leaf

Leaf Area = length of leaf × maximum width of leaf × 0.75 (correction factor)

Biomass assessment: The dry and fresh weights were measured by using electrical balance Sartorius GMBH, type 1216 MP 6E Gottingen, Germany, drying oven (Wiseven, Model WOF-105, Korea) was used to dry the samples. The attributes of biomass noted were Shoot fresh weight (g), Shoot dry weight (g), Root fresh weight (g), Root dry weight (g), Total fresh weight (g) and Total dry weight (g).

Yield assessment: The yield was assessed by measuring following parameters: Leaves fresh weight (g) and Leaves dry weight (g)

Statistical analysis: The data attained were examined by means of one-way ANOVA, via SPSS software. Duncan's multiple range test was applied for partitioning of means for significant treatment at $p \leq 0.05$. The stated values are means of 3 replications ± SE.

RESULTS

Determination of morphological parameters: Reduction in all morphological growth characters of Spinach (*Spinacia oleracea* L.) was noticed by increasing the concentration of Sodium Fluoride. Shoot length displayed a significant gradual decline with increasing the concentration of sodium fluoride applied as soil drench in both first and final harvests from control to 300ppm.

After 44 days after sowing (DAS) the highest shoot length was observed at control, i.e., 21.6cm and shortest shoot length, i.e., 13.27cm was recorded at 300ppm concentration of Sodium fluoride as demonstrated in Table 1. The shoot length gradually decreased with increasing salt (NaF) concentration consequently minimum shoot length was recorded at NaF-300. At lower concentrations, the effect of salinity stress was minor but by further increasing the salt concentration, a noticeable effect on root length could be seen. Plants treated with 50, 100, 150, 200, 250, and 300ppm displayed a prominent decline and the values for root length were 12.07cm, 11.53cm, 10.23cm, 8.9cm, 8.33cm and 7.53cm, respectively (Table 1).

Petiole displayed reducing order when various concentrations of salt, i.e., NaF were given to spinach plants as soil drench. With rising concentrations, length of petiole was shortened. Both harvests followed same tendency of decrease of petiole length with increase in salinity stress.

Table 1. Morphological growth parameters of *Spinacia oleracea* L. harvested at 44 DAS and 60 DAS under 7 treatments of NaF during growth season October, 2019 to December, 2019

Treatments	Parameters (44 DAS)				
	Shoot Lengths (cm)	Root Length (cm)	No. of Leaves	Leaf Area (cm^2)	Petiole Length (cm)
Control	21.6 ±0.37f	11.83±0.21g	27.66±0.23f	69.32 ±0.16g	5.6±0.28e
50ppm	19.6 ±0.25e	11.27±0.13f	21.66±0.33e	51.85±0.04f	5.1±0.23d
100ppm	17.8±0.17d	11.1±0.05e	17.67±0.36d	42.19±0.08e	4.47±0.29cd
150ppm	16.87 ±0.20c	10.23±0.17d	14.67±0.17d	37.3±0.12d	3.67±0.28c
200ppm	15.2 ±0.15b	9.13±0.03c	13.67±0.21c	27.8±0.10c	3.23±0.28b
250ppm	14.17 ±0.17b	8.67±0.21b	10.67±0.19b	15.41±0.15b	2.8±0.15ab
300ppm	13.27 ±0.14a	8.07±0.08a	9.33±0.22a	9.79±0.25a	1.8±0.15a
Treatments	**Parameters (60 DAS)**				
	Shoot Lengths (cm)	**Root Length (cm)**	**No. of Leaves**	**Leaf Area (cm^2)**	**Petiole Length (cm)**
Control	25.93 ±0.26f	13.13±0.21f	47.66±0.33g	103.01 ±0.36g	8.87±0.14g
50ppm	24.23 ±0.24e	12.07±0.20e	41.33±0.21f	88.19±0.11f	8.37±0.14f
100ppm	22.8±0.21e	11.53±0.20d	32.66±0.17e	76.92±0.11e	7.87±0.14e
150ppm	21.13 ±0.20d	10.23±0.17c	24.33±0.26d	66.68±0.18d	7.07±0.14d
200ppm	19.07 ±0.20c	8.9±0.11b	19.33±0.18c	55.07±0.26c	6.4±0.15c
250ppm	18.47 ±0.23b	8.33±0.12b	15.33±0.36b	49.15±0.21b	5.53±0.14b
300ppm	16.23 ±0.24a	7.53±0.20a	10.33±0.19a	41.72±0.10a	4.9±0.11a

Data demonstrate means ± SE of 3 replicates. Non-identical letters depict significant dissimilarity between the treatments ($P \leq 0.05$).

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NaF-50 treated plants had considerably a greater number of leaves than rest of the treated plants but lower number of leaves as compared to control and contained 21.67 leaves by average per pot (Table 1). After 60 DAS lowest number of leaves, i.e., 10.33 by average, were contributed by plants treated with 300ppm NaF solution. As control plants were not harmed by NaF stress that is why after 44 DAS, leaf area was noticed as maximum for control plants, i.e., 69.32cm^2 and the smallest value of leaf area was evident as 9.79cm^2 for 300ppm as described by Table 1. At 60 DAS, decline in leaf area was in a similar fashion as in first harvest.

Biomass assessment: With increase, in the concentration of sodium fluoride, there was a major decline in the fresh weight of shoot. The control plants had highest weight and healthiest as compared to all other treated plants. At 44 DAS and 60 DAS shoot fresh weight was found to be highest for control because of absence of salt stress. Plants treated with 50ppm concentration had greater shoot fresh weight as compared to all other higher salt concentrations treated plants. For 50ppm, 100ppm, 150ppm, 200ppm, 250ppm, and 300ppm, it was 25.3g, 21.77g, 17.37g, 11.63g, 7g, and 4.73g respectively. Percentage reduction had declining trend as 24.8%, 35.9%, 48.9%, 65.8% 79.4%, and 86.1% for 50ppm, 100ppm, 150ppm, 200ppm, 250ppm, and 300ppm respectively (Table 2). It was clearly observed that maximum reduction took place with plants that were treated with 300ppm solution.

Same reducing tendency was observed in root fresh weight as shown by shoot fresh weight. In both first and final harvest control had thicker roots and greatest value of root fresh weight. At 60 DAS, highest root fresh weight, i.e., 4.7g was recorded for control plants with chunkier roots (Table 2). Plants dealt with NaF-50 and NaF-300 are the least and highly affected plants respectively by salt stress. Plants were dealt with 7 different treatments in current experiment and dry weight of shoot as well as root exhibited same reducing trend just like fresh weight. Highest dry weight was owned by control as it was not grown under salt stress. Other salt treated plants came up with an evident reduction in their total dry weight. Maximum reduction in shoot and root dry weight was observed in NaF-300 treated plants as these were grown under highest level of stress.

Yield assessment: Spinach was grown in fall (autumn) season and that is why no inflorescence was observed as it grows in late spring when temperature is higher and other environmental conditions are favorable. So in this experiment yield was taken as Leaves fresh weight (g) and Leaves dry weight (g). Plants treated with NaF-300 had lowest fresh weight value, i.e., 3.5g because these plants were adversely affected by high salt stress (Figure 1). Leaves fresh weight for 50, 100, 150, 200, and 250ppm treated plants was 16.83g, 14.6g, 10.5g, 7.9g, and 4.43g respectively (Figure 1). According to Figure 1, at 60 DAS, control leaves were recorded to be the healthiest and broad with greatest fresh leaves having fresh weight of 46.93g and plants treated with 300ppm solutions had lowest leaves fresh weight value, i.e., 9.4g because these plants were badly affected by high NaF stress. At 44 DAS, control plants were recorded to have highest value of leaves dry weight, i.e., 2.52g and these plants had more weight of dry leaves, i.e., 2.43g than rest of all other greater levels of salinity stress (Figure 2). At 60 DAS lightest weight of dry leaves was for 300ppm.

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Table 2. Biomass assessment of *Spinacia oleracea* L. harvested at 44 DAS(Above) and 60 DAS (below) under 7 treatments of NaF during growth season October, 2019 to December, 2019

Treatments	Parameters (44DAS)					
	Shoot Fresh Weight (g)	Root Fresh Weight (g)	Total Fresh Weight (g)	Shoot Dry Weight (g)	Root Dry Weight (g)	Total Dry Weight (g)
Control	33.97g ±0.12	3.57f ±0.16	37.53g ±0.36	3.53f ±0.20	0.83e ±0.06	4.37f ±0.21
50ppm	25.3f ±0.03	2.87f ±0.07	28.17f ±0.17	3.27e ±0.20	0.7de ±0.06	4e ±0.21
100ppm	21.77e ±0.17	2.17e ±0.06	23.93e ±0.17	2.63d ±0.14	0.63d ±0.05	3.23d ±0.12
150ppm	17.37d ±0.29	1.6d ±0.05	18.97d ±0.34	2.07c ±0.08	0.47c ±0.03	2.53c ±0.12
200ppm	11.63c ±0.29	1.07c ±0.08	12.7c ±0.26	1.47b ±0.14	0.28b ±0.01	1.75b ±0.16
250ppm	7b ± o.27	0.69b ±0.09	7.69b ±0.24	0.9a ±0.11	0.19a ±0.005	1.09a ±0.10
300ppm	4.73a ±0.46	0.7a ±0.005	5.4a ±0.26	0.5a ±0.05	0.62a ±0.01	0.62a ±0.04

Treatments	Parameters (60 DAS)					
	Shoot Fresh Weight (g)	Root Fresh Weight (g)	Total Fresh Weight (g)	Shoot Dry Weight (g)	Root Dry Weight (g)	Total Dry Weight (g)
Control	58.5g ±0.34	4.7g ±0.11	63.1g ±0.29	5.84f ±0.04	0.46f ±0.008	6.3f ±0.04
50ppm	54.03f ±0.12	4.2f ±0.05	58.23f ±0.16	5.75e ±0.32	0.43e ±0.01	6.17e ±0.32
100ppm	43.9e ±0.23	3.5e ±0.11	47.4e ±0.30	4.39d ±0.01	0.35e ±0.008	4.74d ±0.02
150ppm	33.17d ±0.17	2.93d ±0.09	36.1d ±0.25	3.31c ±0.01	0.28d ±0.01	3.59c ±0.02
200ppm	29.33c ±0.23	2.4c ±0.15	37.73c ±0.38	2.91b ±0.03	0.22c ±0.008	3.14b ±0.04
250ppm	22.17b ± 0.17	1.9b ±0.11	24.07b ±0.24	2.22a ±0.02	0.196b ±0.01	2.43a ±0.01
300ppm	17.33a ±0.21	1.33 ±0.08	18.67a ±0.29	1.72a ±0.01	0.123a ±0.008	1.53a ±0.008

Data demonstrate means ± SE of 3 replicates. Non-identical letters depict significant dissimilarity between the treatments ($P \leq 0.05$).

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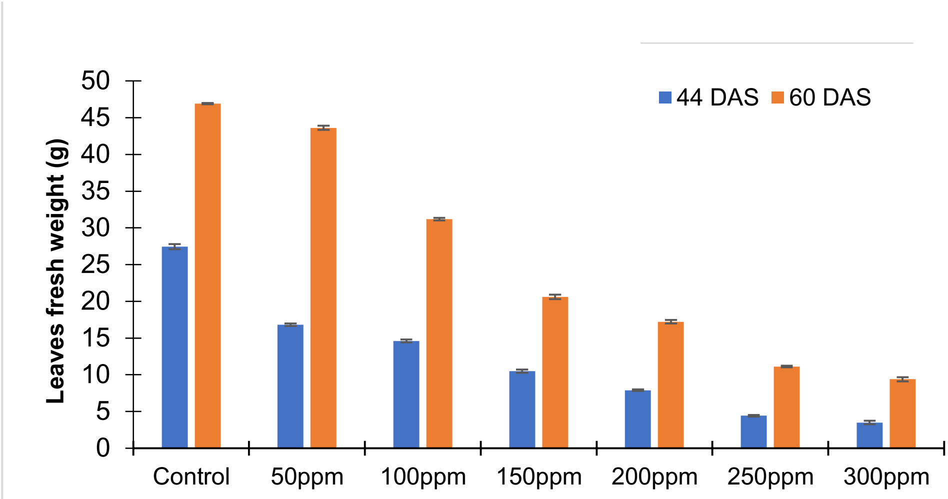

Figure 1. Showing comparison of mean value of Leaves fresh weight of first (44 DAS) and final harvest (60 DAS) in *Spinacia oleracea* L. under 7 treatments of NaF during growth season of October, 2019 to December, 2019.

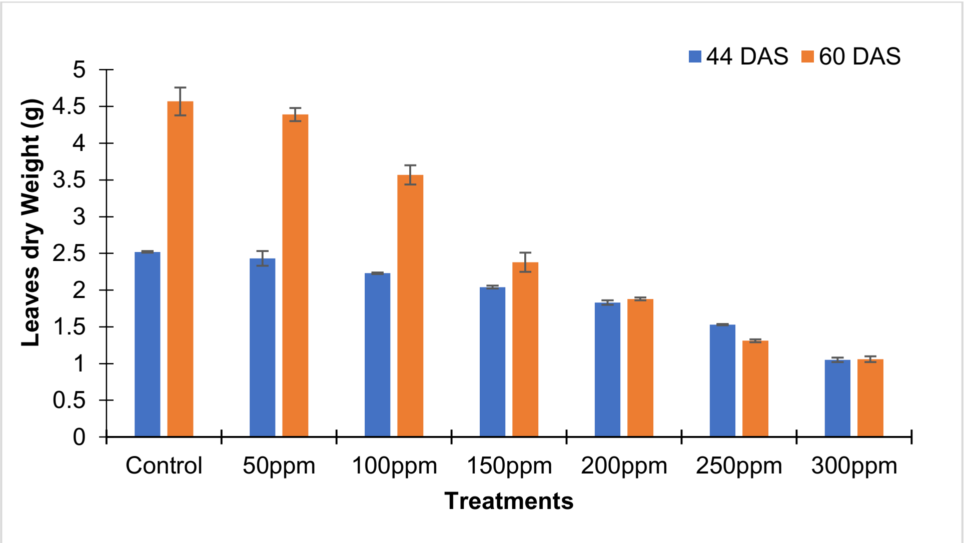

Figure 2. Showing comparison of mean value of Leaves dry weight of first (44 DAS) and final harvest (60 DAS) in *Spinacia oleracea* L. under 7 treatments of NaF during growth season of October, 2019 to December, 2019.

DISCUSSION

Spinach (*Spinacia oleracea* L.) is extensively grown in Pakistan where salinity is one of the major and deleterious issue to the growth of crops. The recent experiment was carried out to investigate the effect of sodium fluoride on the growth, and yield characters of spinach. It was found that the vegetative growth parameters of *Spinacia oleracea* L. exhibited significant dose-related decline with elevating NaF concentrations.

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We could clearly observe a gradual, linear reduction pattern in different morphological characters, when the concentration of salt was increased linearly. Shoot and root length did not show any significant reduction at lower concentration, i.e., 50ppm, but there was a prominent decline when gradually moved to higher concentrations. The reduction in length of shoot was more noticeable than root length. It showed greater and prominent effect of salt stress on growth of shoot. Root and shoot length reductions in current results are in accordance with the findings by many other researchers in previous studies with *Lycopersicum esculentum* [27], *Brassica rapa* [28,29], *Cicer arieninum* [30]. They found that plants exposed to increasing levels of NaF suffered reduction in root and shoot length and thus their weight.

Ors and Suarez [24] clearly demonstrated that supplementary salinity stress aggravated the decline in yield. In findings of the recent study leaves fresh weight and leaves dry weight reduced with elevating the level of salt stress. Zuo et al. [31] observed the tendency of fluorides to get accumulate in the leafy portions of some vegetables which badly affected pattern of growth and crop productivity. Saroj et al. [32] reported biomass reduction in *Vigna aconitifolia* L. due to saline stress. Sabal et al. [33] also reported similar decline in biomass of plants. The current study is also a significant example of similar results.

Because of reduction in length and width, leaf area in recent study is also reduced which is related to finding of Mehta et al. [34] who reported decline in leaf area because of high salt accumulation. Jamil et al. [35] observed that leaf number and leaf area were harshly damaged with saline treatment of plants. Gama et al. [36] also registered that increased F concentration was responsible for lessening of number of leaves. Zhao et al. [37] also provided the supporting fact about declining leaf area with increasing salinity concentration due to reduction in leaf length. Results of present studies with respect to leaf number and leaf area was also related to these studies. Significant reduction in leaf number and leaf area was witnessed at higher saline concentrations, i.e., at 200ppm to 300ppm.

Zouari et al. [20] investigated the effect of sodium fluoride on young olive trees and noticed the fact that high concentration caused pronounced effects whereas plants displayed a little tolerance to F stress at lower concentrations of sodium fluoride. Same was the case with recent study, where high salt concentration affected more severely. So, this study demonstrated the sensitivity of Spinach in Fluoride polluted soil, which was detrimentally affected by the application of NaF as soil drench and exhibited considerable reduction in yield, biomass, and other morphological parameters.

CONCLUSIONS

Recent experiment unveiled the toxic effects of different concentrations of salt under field conditions. Fluoride caused deleterious effects on yield characteristics, morphological growth parameters, and biomass attributes. Responses shown by stressed plants showed tremendous reduction in growth parameters in both destructive harvests at 44 DAS and 60 DAS. Plant biomass was reduced as a result of increasing stress. On the other hand, control plants flourished well and produced more biomass. So, it can clearly be inferred that Spinach is Fluoride sensitive and can

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not grow well in F contaminated soil. In Pakistan soils that are contaminated with a heavy, toxic load of NaF that is why removal of high levels of sodium fluoride from soil is the need of the hour in irrigated lands so that its yield could be increased in future.

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